Nucleic Acid Based Diagnostic Methods for Viral Assays

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Summary: Diagnostic Methods in Virology

- Direct Examination
  - Bright Field Microscopy for viral CPE- histopathological changes
  - Electron microscopy for virus morphology
  - Fluorescent Microscopy- Immunofluorescence for Viral Antigen imaging
  - Hybridization of viral genomes- FISH
- Indirect/Infectivity Assays
  - Cell Culture methods- plaque, CPE
  - Embyonated eggs: Haemagglutination, POCK, Inclusion bodies
  - Experimental Animal models- Disease/ death

Summary: Diagnostic Methods in Virology

- Serological Methods
- Classical-
  - Complement Fixation
  - Haemagglutination Inhibition Assay
  - Neutralization Assay
  - Single Radial Hemoysis
- Advanced
  - Immunoassays: ELISA, RIA
  - Western Blotting

Molecular Testing

Technique	Format	Examples of viruses
NON AMPLIFIED NUCLEIC ACID PROBES	Liquid-phase Solid-phase *In situ* hybridization	HPV, CMV
AMPLIFIED NUCLEIC ACID TECHNIQUES		
Signal amplification techniques	bDNA assays Hybrid capture assays	HCV, HBV, HIV HPV, CMV
Target amplification techniques	PCR techniques RT-PCR Nested PCR Multiplex PCR Real-time PCR Transcriptional-based amplification methods Strand displacement amplification LAMP HDA	Most of viruses RNA virus (HCV, HIV) Herpesviruses Herpesviruses, respiratory viruses Most of viruses HCV (TMA-based), HIV (TMA-based, NASBA), CMV (NASBA) Enterovirus (NASBA), RSV (NASBA) HIV Influenza A and B, CMV, HSV, VZV, BK virus, HPV HIV-1, HSV 1 and 2
Probe amplification techniques	Ligase chain reaction Cycling probe technology Cleavase-invader technology	 HCV, HPV
MICROARRAYS	DNA microarrays Multiplexed microsphere-based array	Respiratory viruses, HCV, HPV, HIV, CNS infection viruses HIV, HCV, HSV

HPV: human papillomavirus; **CMV:** cytomegalovirus; **HCV:** hepatitis C virus; **HBV:** hepatitis B virus; **HIV:** human immunodeficiency virus; **TMA:** transcription-mediated amplification; **NASBA:** nucleic acid sequence-based amplification; **RSV:** respiratory syncytial virus; **DNA:** deoxyribonucleic acid; **RNA:** ribonucleic acid; **LAMP:** loop-mediated isothermal amplification; **HDA:** helicase-dependent amplification.

Cobo, 2012

Amplified nucleic acid techniques

Signal amplification techniques

- bDNA assays
- hybrid capture assays

Target amplification techniques

- PCR techniques
- transcription-based amplification methods
- strand displacement amplification

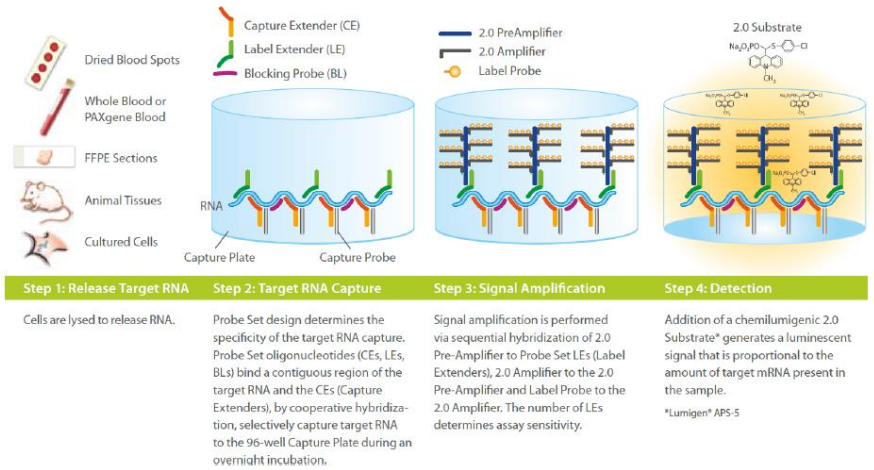
Probe amplification techniques

- ligase chain reaction
- cycling probe technology

Hybridization Techniques

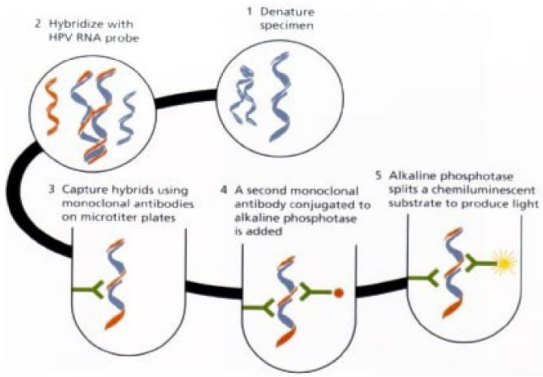
- A branched DNA (bDNA) assay is a signal amplification technology that detects nucleic acid molecules.
- It's also known as the sandwich nucleic acid hybridization method.

bDNA (branched DNA) assays



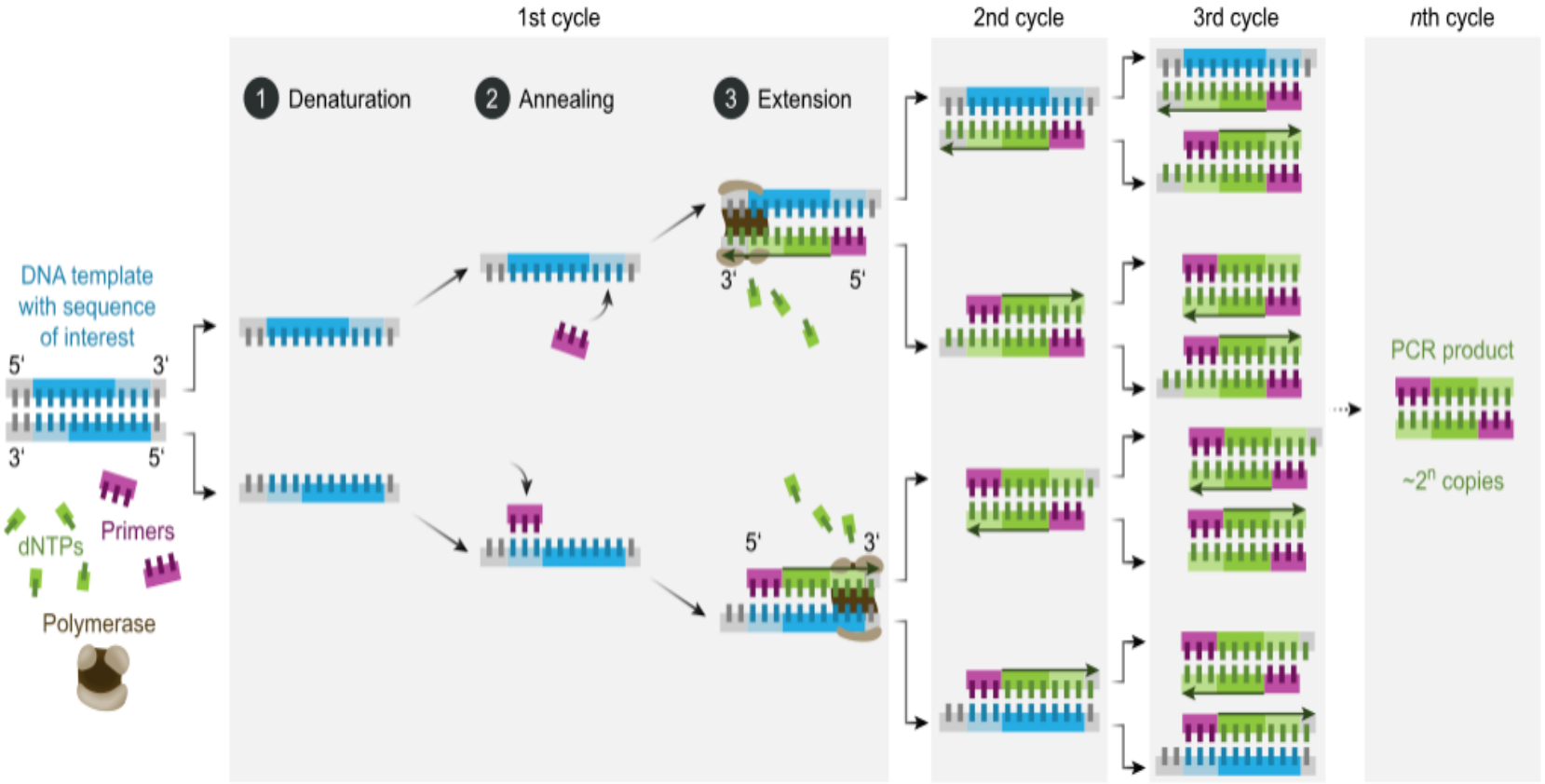
- The signal is proportional to the number of labeled probes
- Commercially available assayes (Bayer HealthCare, Diagnostic Division, Tarrytown, N.Y.)
- HCV, HBV, HIV-1

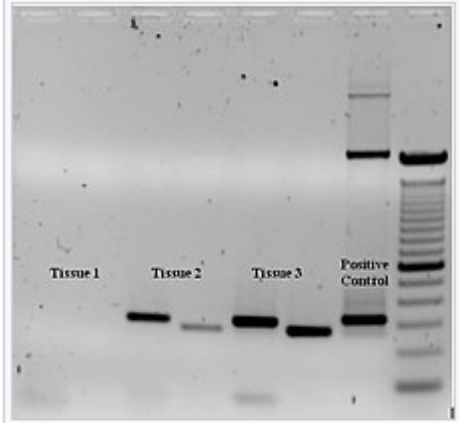
Hybrid Capture Assays



- RNA/DNA hybrid molecule
- Anti-hybrid antibody (capture), anti-hybrid detection antibody (labeled)
- Commercially available assays: Digene Corp. (Gaithersburg, Maryland, USA): HPV, CMV, Chlamydia, Neisseeria

PCR (Wikipedia)



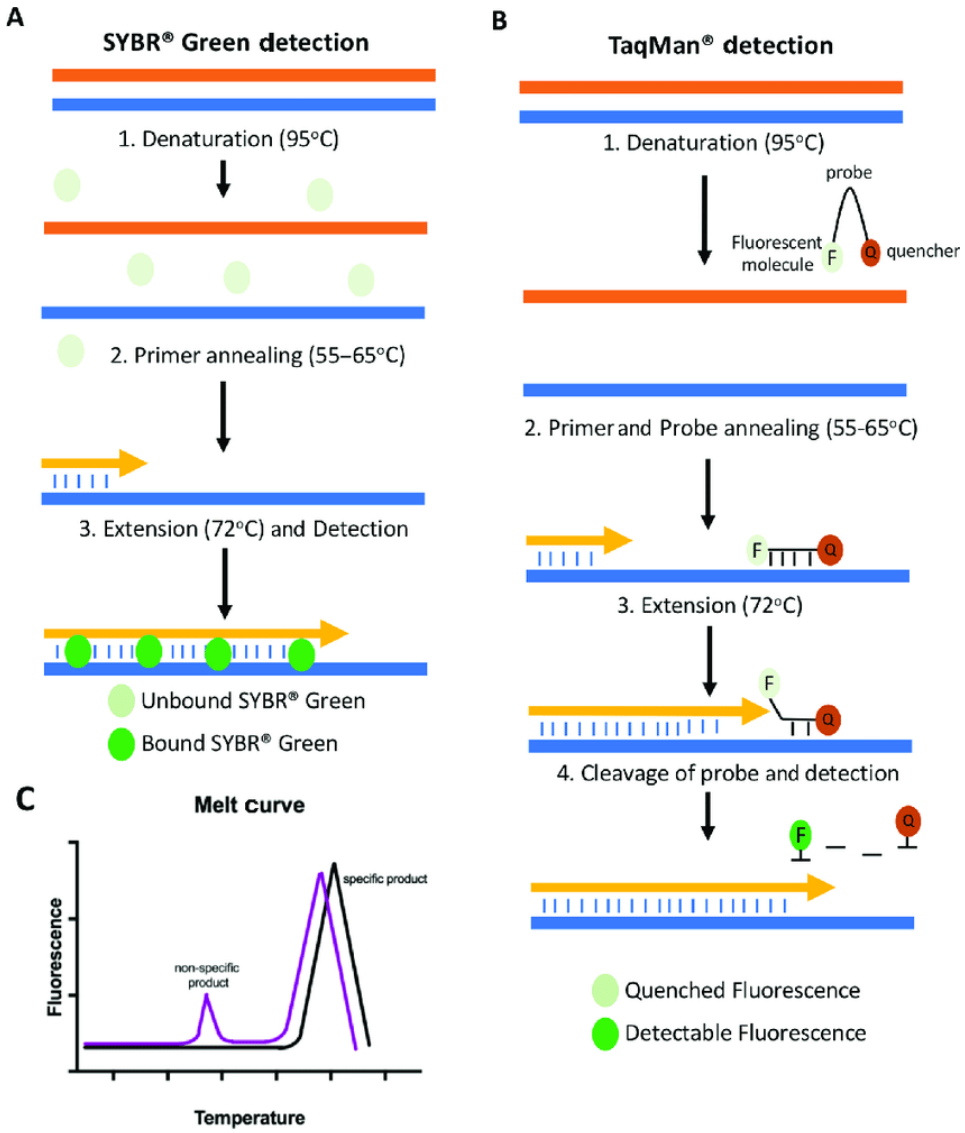


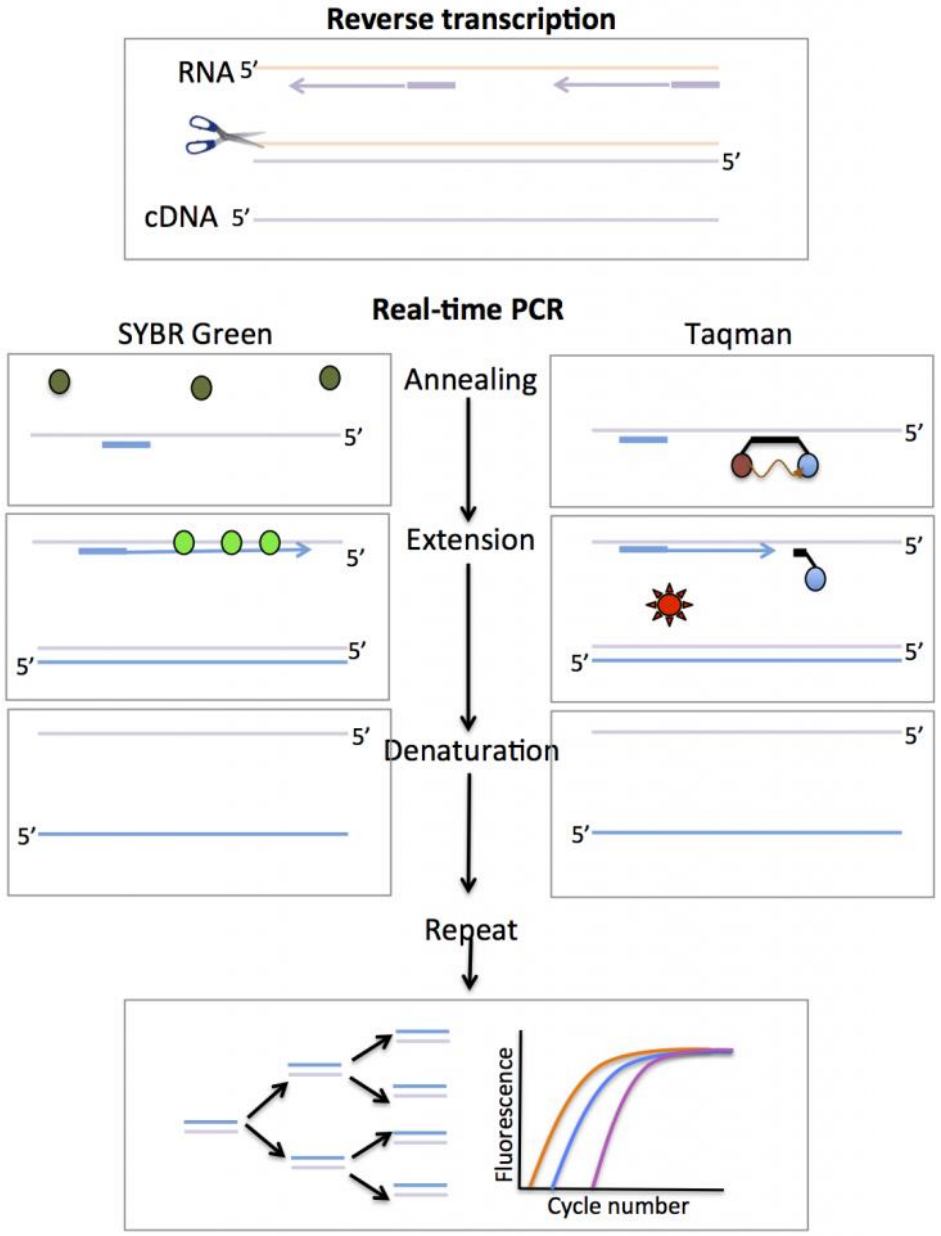
Ethidium bromide-stained PCR products after gel electrophoresis. Two sets of primers were used to amplify a target sequence from three different tissue samples. No amplification is present in sample #1; DNA bands in sample #2 and #3 indicate successful amplification of the target sequence. The gel also shows a positive control, and a DNA ladder containing DNA fragments of defined length for sizing the bands in the experimental PCRs.

PCR techniques

- **Reverse transcriptase-PCR**
- • RNA transcription into cDNA
- • retroviral reverse transcriptases or thermostable Tth DNA-polymerase
- • commercially available kits: HCV, HIV-1 in clinical specimens
- **Nested-PCR**
- • 2 pairs of amplification primers
- • increased sensitivity and specificity
- **Multiplex PCR**
- • 2 or more primer sets
- • more than one target sequence co-amplified
- • commercial kits: viruses of respiratory and central nervous system
- **Real time PCR/Quantitative real time PCR**
- • starget amplification and detection steps are simultaneous
- • software-based monitoring the data at every cycle - quantification

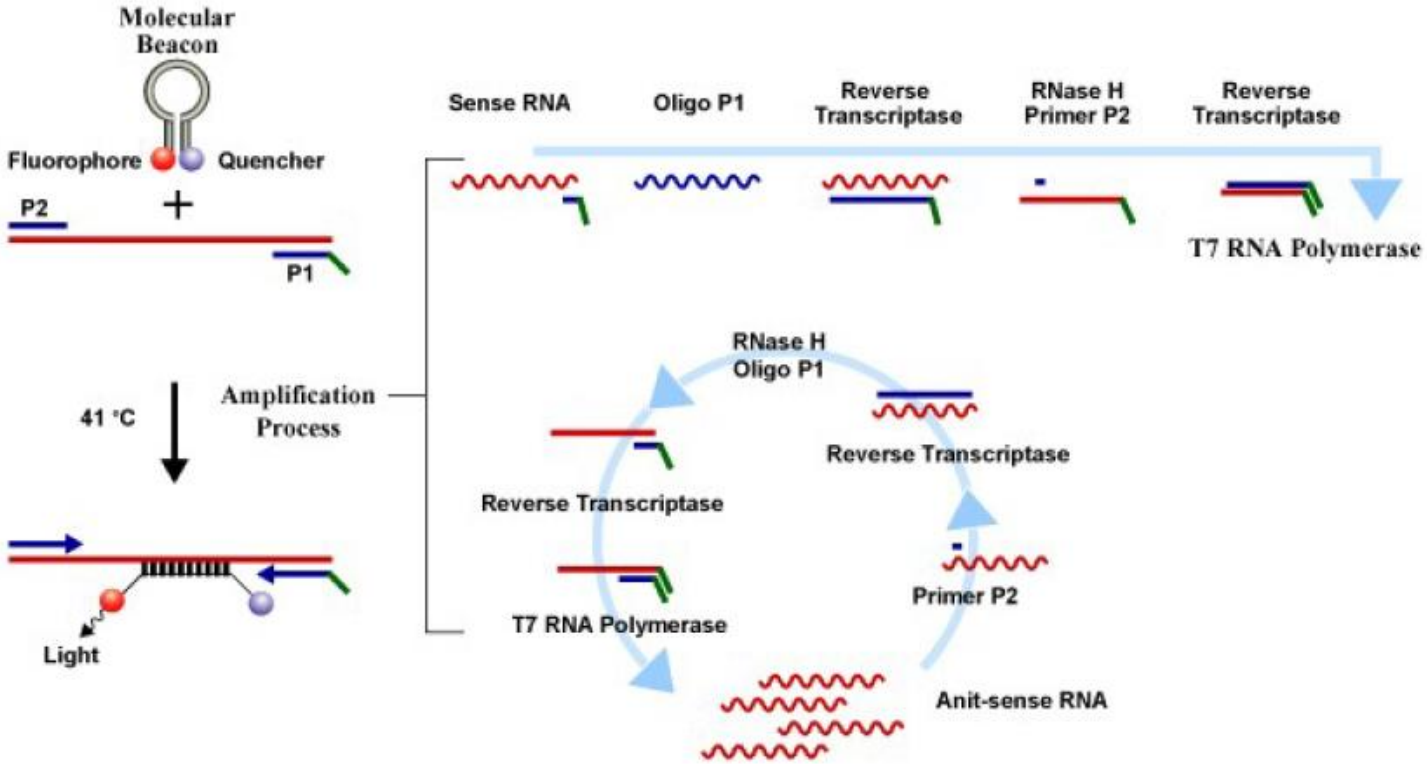
Real Time PCR





A beginner's guide to RT-PCR, qPCR and RT-qPCR - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/Comparison-of-intercalating-dye-and-hydrolysis-based-probe-detection-A-SYBR-R-Green_fig3_342182497 [

Nucleic acid sequence-based amplification (NASBA)



- isothermal RNA amplification method
- avian myeloblastosis virus RT, RNase-H, T7-RNA polymerase
- no requirement for a thermal cycler, rapid kinetics, ssRNA-no denaturation prior deteiction
- bioMérieux: HIV-1, CMV, enterovirus, respiratory syncytial virus
  West Nile virus, St. Louis encephalitis, Dengue virus

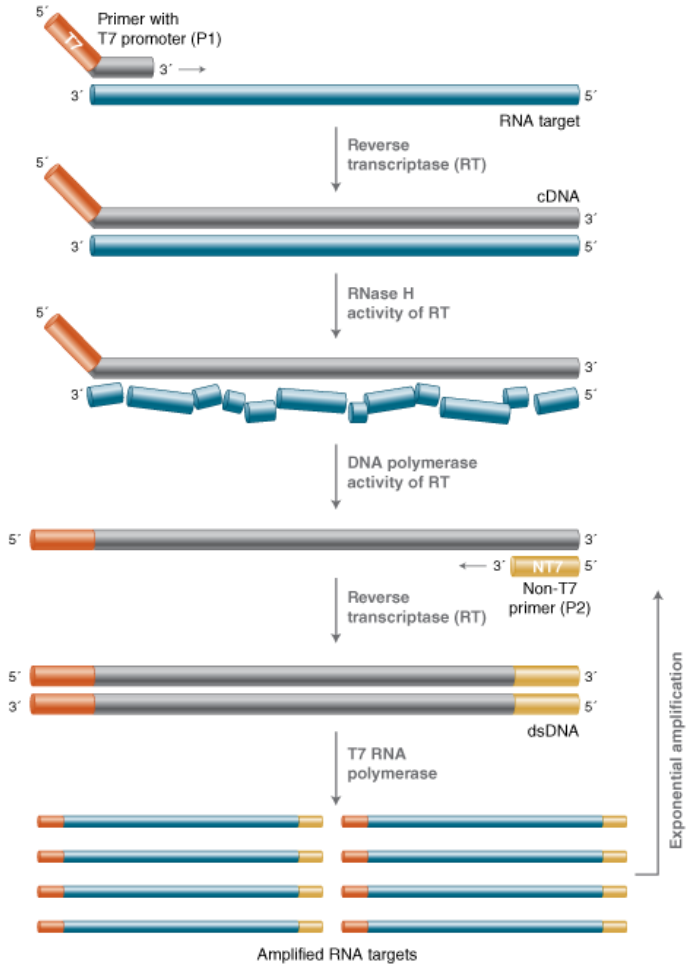
Nucleic acid sequence based amplification (NASBA) is a used to amplify RNA sequences. RNA template is given to the reaction mixture, the first primer attaches to its complementary site at the 3′ end of the template.

Isothermal Amplification Methods

- A low-cost alternative to detect certain diseases that was invented in 2000 at the University of Tokyo
- Isothermal amplification is a nucleic acid amplification technique that doesn't require temperature changes to separate DNA strands. This eliminates the need for a thermocycling machine
- Performed in single uniform temperature in a heating block/water bath
- Loop mediated isothermal amplification- Dengue,SARS, Influenza, Herpes
- 2004.Vincent Helicase Displacement Amplification is one of the simplest approaches for isothermal nucleic acid amplification that mimics an in vivo process of DNA replication, using a helicase to isothermally unwind DNA duplexes instead of heat, as is the case in PCR.

NASBA: Nucleic Acid Sequence Based Amplification (Wikipedia)

- NASBA is a two-step process that takes RNA and anneals specially designed primers, then utilizes an enzyme cocktail to amplify it
- The NASBA technique has been used to develop rapid diagnostic tests for several pathogenic viruses with single-stranded RNA genomes, e.g. influenza A, zika virus, foot-and-mouth disease virus, severe acute respiratory syndrome (SARS)-associated coronavirus



https://www.neb.com/en/applications/dna-amplification-pcr-and-qpcr/isothermal-amplification/nucleic-acid-sequenced-based-amplification-and-transcription-mediated-amplification

Different types of Isothermal Amplification Methods

Property	PCR	NASBA	SMART	SDA	RCA	LAMP	HDA	SPIA
DNA amplification	+	+	+	+	+	+	+	+
RNA amplification	+ (RT*-PCR)	+	+	+ (RT*-SDA)	+ (RT*-RCA)	+ (RT*-LAMP)	+ (RT*-HDA)	+
Temperature(s)°C	94, 55–60, 72	37–42	41	37	37	60–65	Room**, 37, 60–65	45, 50
Number of enzyme(s)	1	2–3	2–3	2	1	1	2	3
Primer design	Simple	Simple	Complex	Complex	Simple	Complex	Simple	Simple
Multiplex amplification	+	+	-	-	-	-	+	-
Product detection method	Gel electrophoresis, ELISA, real-time	Gel electrophoresis, ELISA, real-time, ECL	ELOSA, real-time	Gel electrophoresis, real-time	Gel electrophoresis	Gel electrophoresis, turbidity, real-time	Gel electrophoresis, ELISA, real-time	Bioanalyzer
Portable test designing	-	+	+	+	-	+	+	+
Tolerance to biological components	-	-	-	-	-	+	+	-
Need to template denaturation	+	+	+	+	-	-	-	+
Denaturing agent(s)	Heat	RnaseH, DMSO	RNaseH	Restriction enzymes, bumper primers	Strand-displacement property of Φ29 DNA polymerase	Btaine	Helicase	RNaseH

*RT=reverse transcriptase; **Room=22–24°C; ELISA=enzyme-linked immunosorbent assay; ELOSA=enzyme-linked oligosorbent assay; ECL=electrochemiluminescence

continuous concatemers of the template [Figure 10]. The these isothermal amplification methods have weaknesses

Karami, Ali & Gill, Pooria & Motamedi, Mohammad & Saghafinia, Masoud. (2013). J GlobalInfectDis 2011 3 3 293 83538.

Post Amplification analyses

- Sequencing

(identification of unknown viruses, identification of resistance

- mutations, HCV/HBV genotyping, HIV drug resistance testing for

monitoring treatment...)

- Luminex analysis

(Multiplexed microsphere-based array, combination of multiplex

- PCR and flow cytometry)
- Nucleic acid arrays

(DNA microarrays)

- Mass spectrometry

(protein expression/proteome analysis)

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- Karami, Ali & Gill, Pooria & Motamedi, Mohammad & Saghafinia, Masoud. (2013). J Global Infect Dis 2011 3 3 293 83538.
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- wikipedia